REMARKS

A check for the fees for a three month extension of time and for filing an RCE accompanies this response. Any additional fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 06-1050.

Claims 1-16 and 30 are pending in this application. Claim 1 is amended to more particularly point out the claimed subject matter. Basis for the amendment can be found throughout the specification, for example, at page 34, lines 8-13, at page 47, lines 18-20, page 51, lines 9-10, page 52, lines 29-31 and page 54, lines 4-6. New Claim 33 is added. Basis for this claim can be found for example, at page 33, lines 3-30, and Example 7 at page 52.

A Supplemental Information Disclosure Statement accompanies this Preliminary Amendment and RCE.

The response filed June 16, 2003, responsive to the previous Office Action, is incorporated by reference herein.

THE REJECTION OF CLAIMS 1-4, 6-16 and 30 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 1-4, 6-16 and 30 are rejected under 35 U.S.C. § 112, first paragraph, because it is alleged that the specification, while being enabling for a method comprising introducing a large nucleic acid molecule metabolically labeled with bromodeoxyuridine or iododeoxyuridine into cells, detecting labeled cells and measuring the product of a reporter gene encoded by the nucleic acid, does not reasonably provide enablement for the method wherein the nucleic acid is labeled by any means.

In consideration of the previous response, filed June 16, 2003, and the DECLARATION of Sandra Vanderbyl, the Examiner has withdrawn the enablement rejection to the extent that the claims are limited to introducing a large nucleic acid molecule metabolically labeled with bromodeoxyuridine or

iododeoxyuridine. The Office Action alleges, however, that the remaining claims encompass introducing nucleic acids labeled by a wide variety of methods, many of which are alleged to damage nucleic acids such that they are no longer competent to express.

This rejection is respectfully traversed.

Relevant law

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples **or** by broad terminology." In re Marzocchi et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

Analysis

1. Summary of Points addressed

The specification teaches one of skill in the art methods for detecting or determining delivery and expression of a nucleic acid introduced into a cell. The methods involve introducing intact and condensed labelled large nucleic acid molecules that encode a reporter gene into cells, such that the delivered nucleic acid molecules remain intact and condensed after delivery, detecting labelled cells as an indication of delivery of the nucleic acid into a cell, and measuring the product of the reporter gene as an indication of DNA expression in the cell.

Applicant respectfully submits that it would not require undue experimentation to practice the full scope of the methods as claimed, namely, to (i) introduce intact and condensed labelled large nucleic acid molecules encoding a reporter gene into cells such that the nucleic acid molecules remain intact and condensed after delivery (ii) detect labelled cells as an indication of delivery of the nucleic acid and (iii) measure the product of the reporter gene as an indication of expression. As discussed below, the specification teaches the steps of the method and the combination of the steps to introduce intact and

condensed labelled large nucleic acid molecules into cells and measure delivery of nucleic acid and gene expression.

As the Examiner has acknowledged, each of the steps/reagents of the instantly claimed methods, *e.g.*, methods for delivering nucleic acids and delivery agents suitable therefor, methods of labeling nucleic acids, were routine and known to those of skill in the art at the time the instant application was filed. Therefore, it is respectfully submitted that those of skill in the art could, by virtue of the teachings of this application, readily practice what is claimed by substituting another delivery agent or another nucleic acid label in the steps of the methods as instantly claimed. It would be unfair and unduly limiting to require Applicant to limit these claims to the use of one or two labels, *e.g.*, BrdU or IdU, for labeling the nucleic acids that are introduced into cells according to the instantly claimed methods.

The Examiner alleges that although each step of the instantly claimed methods may employ methods and reagents that were known to those of skill in the art at the time the instant application was filed, it is the combination of steps that is not enabled for its full scope because the step of labeling can allegedly damage nucleic acids such that they are no longer competent to express.

In response, as discussed in detail below, it is respectfully submitted that the Examiner has failed to establish that obtaining gene expression from labelled nucleic acids was unpredictable or inoperable at the time the instant application was filed. To the contrary, Applicant has provided several references demonstrating knowledge in the art, at the time the instant application was filed, with respect to labeling nucleic acids in a manner that does not disrupt gene expression.

Additionally, the DECLARATION of Sandra Vanderbyl submitted with the previous response filed June 16, 2003, which is incorporated by reference herein, demonstrates operability of the methods, including the combination of

the steps of the methods, and also further evidences enablement of the claims as of their effective filing date. The DECLARATION submitted with the previous response clearly demonstrates that labeled large DNA molecules can be delivered into and expressed in cells. The DECLARATION also demonstrates that the presence of the label does not interfere with gene expression. Therefore, by following the methods provided in the specification, one of skill in the art can deliver into and express labeled large nucleic acid molecules in cells. The law does not require demonstration of delivery and expression of large nucleic acids containing every possible label incorporated into the nucleic acids by every possible method, as long as Applicant has demonstrated how to carry out the steps of the method as claimed.

Further, at the time of filing of the instant application, a variety of labelling methods compatible with gene expression were available in the art for use with the claimed methods. As discussed in detail below, Applicant has provided several references demonstrating the knowledge in the art at the time of filing of methods for labelling DNA that do not disrupt gene expression. Thus, given the state of the art and the teachings of the specification, Applicant respectfully submits that the scope of the claimed methods are commensurate with the teachings of the specification.

2. Application of the factors enumerated in Ex parte Forman

As noted above, the test for scope of enablement is whether the specification teaches one of skill in the art to make and use what is claimed without undue experimentation. The courts have enumerated a number of factors to be considered in assessing whether experimentation is undue.

Scope of the claims

The claims are directed to methods for detecting or determining delivery and expression of a nucleic acid introduced into a cell by:

introducing condensed and intact labelled large nucleic acid molecules that encode a reporter gene into cells, where the nucleic acid molecules are

intact and condensed after delivery; detecting labelled cells as an indication of delivery of the nucleic acid into a cell; and measuring the product of the reporter gene as an indication of DNA expression in the cell. The dependent claims further specify methods of detection, types of nucleic acids and cells, delivery methods and labelling methods.

Level of Skill

As discussed in the previous response of June 16, 2003, which is incorporated by reference herein, and as acknowledged in the instant Final Office Action of September 30, 2003, the level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). Further, the numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art.

Teachings of the specification

The instant application teaches the delivery of nucleic acids, including labelled nucleic acid molecules, into cells and the expression of gene products encoded therein. The teachings of the specification describe how to: introduce intact and condensed labelled large nucleic acid to a cell such that the nucleic acid molecules remain intact and condensed after delivery; measure the delivery of the nucleic acid to the cell by detecting labelled cells; and determine the expression of the product of the reporter gene encoded by the nucleic acid.

The instant application teaches the combination of delivering labelled nucleic acid molecules and detecting gene expression (*see*, for example, Example 7, pages 52-57). The specification exemplifies methods of detecting gene expression from labeled DNA for two labels, bromodeoxyuridine and iododeoxyuridine, and two reporter genes, β-galactosidase and GFP. The specification demonstrates labelled DNA delivery and expression in several cell types and with a variety of DNA delivery agents. The instant application also teaches how to prepare intact and condensed large labelled nucleic acid (*see*,

for example, page 52, lines 10-31) and how to assess whether nucleic acid molecules remain intact and condensed before and after delivery to a cell (*see*, for example, page 34, lines 4-14, page 43, lines 27-31, and page 54, lines 4-6). Further, the application demonstrates the combination of the steps of the method to determine delivery and gene expression from intact and condensed labelled large nucleic acid molecules (Example 7, pages 52-57).

As acknowledged by the Examiner, the DECLARATION under 37 C.F.R. § 1.132 of Sandra Vanderbyl submitted June 16, 2003, demonstrates that by following the steps of the instant methods according to the teachings of the specification, one of skill in the art can obtain gene expression from intact and condensed labelled large nucleic acid molecules delivered into cells at an efficiency that is comparable to that of unlabelled DNA. Thus, Applicant has demonstrated that the application teaches labeling and detection of gene (see, *e.g.*, the DECLARATION of Sandra Vanderbyl). Although the DECLARATION was submitted to rebut the Examiner's assertions of inoperativeness, it also further evidences enablement.

It is noted that the level of skill in the biotechnical arts is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Further, methods for performing the various steps of the claimed methods, such as labelling nucleic acids, delivering nucleic acids to cells and measuring delivery of and gene expression from the delivered nucleic acids are known to the skilled artisan.

The Office Action alleges that Applicant has not enabled the full scope of the claims. Applicant respectfully submits that it is not required to provide data or illustrative examples in support of everything within the scope of a broad claim. In re Anderson, 176 USPQ 331, at 333 (CCPA 1973). Further, as long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. is satisfied. In re Fisher,

427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The specification exemplifies the methods with bromodeoxyuridine and iododeoxyuridine labelled large nucleic acid. Applicant further teaches how to prepare and maintain intact and condensed labelled large nucleic acids both before and after delivery to cells; measure the delivery of the nucleic acid to the cell by detecting labelled cells; and determine the expression of the product of the reporter gene encoded by the nucleic acid. These teachings are not limited by the nucleic acid labelling method. As will be further discussed below, given the knowledge in the art of several labelling methods compatible with gene expression, the teachings of the instant application are illustrative of the full scope of the claims.

Knowledge of those of skill in the art

The instant application details their application to the claimed and disclosed methods, which are directed to methods for delivering large intact and condensed labelled nucleic acids and detection of expression. Reagents for performing the steps of the methods were known and available at the time of the effective filing date of the instant claims.

As discussed in the response filed June 16, 2003, which is incorporated by reference herein, at the time of filing of the application, a broad body of knowledge had amassed in the areas of delivery of nucleic acids to cells and in the use of reporter genes and numerous such procedures are referenced in the instant application. These references are directed to agents and instruments to aid in the delivery of nucleic acid molecules and sources therefor (*e.g.*, U.S. Patent Nos. 6,027,488, 5,993,434, 5,944,710, 5,507,724, 5,501,662, 5,389,069, 5,318,515 (page 23, lines 20-26); procedures and methods for ultrasound systems (International PCT application No. WO 99/21584 and U.S. Patent No. 5,676,151 (page 22, line 10 through page 23, line 2); delivery agents such as cationic reagents such as Lipofectin, LipofectAMINE, and LipofectAMINE PLUS from Life Technologies, Inc., (Burlington, Ont., *see* U.S. Patent No. 5,334,761 and 5,736,392; *see, also* U.S. Patent No. 6,051,429),

Effectene (Qiagen, Inc., Mississauga, Ontario) (Non liposomal lipid formulation), Metafectene (Biontex, Munich, Germany) (Polycationic lipid), Eu-fectins (Promega Biosciences, Inc., San Luis Obispo, CA) and numerous other cationic lipids (page 20, line 24 through page 21, line 18), as well as non-lipid compounds such as SUPERFECT™ (Activated dendrimer (cationic polymer:charged amino groups); Qiagen, Inc., Mississauga, ON) and CLONfectin™ (Cationic amphiphile N-t-butyl-N'-tetradecyl-3-tetradecyl-aminopropionamidine; Clontech, Palo Alto, CA) (page 21, line 20 through page 22, line 4); procedures relating to the use of reporter genes (Alton and Vapnek (1979), *Nature 282*: 864-869) luciferase, *e.g.*, firefly luciferase (deWet *et al.* (1987), *Mol. Cell. Biol. 7*: 725-737); bacterial luciferase (Engebrecht and Silverman (1984), *PNAS 1*: 4154-4158; Baldwin *et al.* (1984), *Biochemistry 23*: 3663-3667); and alkaline phosphatase (Toh *et al.* (1989) *Eur. J. Biochem. 182*: 231-238, Hall *et al.* (1983), *J. Mol. Appl. Gen. 2*: 101) (page 16, lines 12-23); and agents for detection of labelled nucleic acids (Pittman *et al.*, *J Immunol Methods 103*: 87-92 (1987) (page 28, lines 16-20) and Gratzer *et al.* Cytometry (1981) 6:385-393 (page 55, lines 8-9).

These references to numerous published protocols for the labelling of DNA and detection thereof, agents for delivery of DNA into cells that can be used in the instantly claimed and disclosed methods, and analysis thereof for expression of encoded genes, including reporter gene products, demonstrate the large volume of information regarding tested and reliable agents available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time.

Additionally, a number of labeling methods for nucleic acids compatible with gene expression were available in the art. For example, the labeling technique of Leahy *et al.* (*Bioconjugate Chem.* (1996) 7:545-551), Neves, Byk, Scherman and Wils (*FEBS Letters* (1999) 453: 41-45; to avoid confusion with the Neves *et al.* reference cited by the Examiner, the FEBS letter reference is

referred to hereinafter as Wils *et al.*) and Zabner *et al.* (*J. Biol. Chem.* (1995) 270:18997-19007) were available as of the filing date of the instant application, in addition to the methods of Felgner *et al.* and Zelphati *et al.*, cited by the Examiner.

Each of the above references demonstrates the successful detection of gene expression from labelled nucleic acids. In Leahy *et al.*, plasmid DNA is labeled with photoactivated biotin (column 1, first full paragraph page 546). The authors demonstrate reporter expression from biotin-labeled plasmid DNA equivalent to unlabeled DNA when the plasmid is labeled such that the transcription cassette is biotin-free (see for example, Figure 6, page 548). Wils *et al.* demonstrates that a psoralen-oligonucleotide-peptide conjugate modified plasmid DNA produced a high level of gene expression that was not diminished as compared to the unmodified plasmid (*see*, for example, Figure 3, page 43). Additionally, Zabner *et al.* demonstrates gene expression with ethidium labeled DNA. Further, as acknowledged by the Examiner, Felgner *et al.* and Zelphati *et al.* each provide a successful method for labelling plasmid DNA using peptide nucleic acid. Thus, at the time of filing of the instant application, the state of the art included many methods for labeling DNA, including chemical labeling of DNA, which did not interfere with gene expression. One of skill in the art could use the teachings of the instant application to apply such labeling techniques for use with the claimed methods for introducing labelled large nucleic acids into cells and measuring DNA delivery and gene expression.

Presence of Working Examples

The Application demonstrates the steps of the claimed methods. Each of the examples exemplifies elements of the claimed subject matter, for example, that intact and condensed large nucleic acids can be labelled in a manner that maintains their intact and condensed structure, large labelled nucleic acids can be introduced into cells and reporter gene activity as well as nucleic acid delivery can be detected or determined. Examples 1, 4, 5, 6, and 7 of the

application describe DNA delivery, measurement of labelled cells and measurement of reporter gene activity. Example 7 further exemplifies all of the elements combined and shows that labelled large nucleic acid can be delivered to cells and DNA delivery and gene expression detected therein.

The working examples also exemplify delivery of intact and condensed labelled large nucleic acids where the nucleic acid remains intact and condensed after delivery. For example, the specification teaches the use of condensing agents to provide intact and condensed DNA for introduction into cells. Hexylene glycol, spermine and spermidine are used in Example 1 in sheath buffer (page 43, lines 27-31) to maintain condensed chromosomes. Similarly, in Example 7, condensing agents were added to maintain intact and condensed chromosomes for introduction into cells (*see*, for example, page 52, lines 39-31).

In addition, the instant application exemplifies methods for identifying intact and condensed DNA, including labelled DNAs, following delivery. Use of FISH to determine intact chromosome delivery is taught for example, in Example 4, page 47, lines 18-20. Similarly, Example 6 exemplifies delivery of chromosomes and the use of FISH to detect intact chromosomes (see for example, page 51, lines 9-10). Example 7 also exemplifies the use of FISH to detect intact labelled chromosomes after delivery (page 54, lines 4-6).

Predictability

The Office Action has predicated the assertion of unpredictability on a broad generalization that labeling of DNA causes DNA damage that impacts the ability to obtain expression of genes encoded by the DNA and that when viewed as a whole, the state of the art allegedly teaches that labelling DNA generally disrupts transcription. The Office Action asserts that this generalization is based on the state of the art as evidenced by Felgner *et al.* and Zelphati *et al.*, and by the presence of inoperative embodiments such as those allegedly taught by Neves *et al.*

The state of the art does not evidence the unpredictability of obtaining gene expression from labelled DNA

Although Felgner *et al.* and Zelphati *et al.* each demonstrate a successful method for labeling plasmid DNA with peptide nucleic acid and obtaining gene expression, the Office Action alleges unpredictability of the state of the art at the time the instant application was filed, based on introductory statements made in these references. For example, the Office Action points to a statement of Felgner *et al.*, "All of the technologies discussed above for chemically modifying plasmid DNA result in DNA damage and interfere with its transcriptional activity" (page 2, line 36 through page 3, line 2).

First, it is noted that Felgner *et al.* and the other references relied upon does not evidence the state of the art at the time of filing of the instant application. Hence, such disclosure is not relevant. Further, the "technologies discussed above" that are referred to in Felgner *et al.* include citations to a number of references: Loyter *et al.* (*Proc. Natl. Acad. Sci.* (1982) 79:422-26), Tsuchiya *et al.* (*J. Bact.* (1988) 170:547-51), Zabner *et al.* (*J. Biol. Chem.* (1995) 270:18997-19007), Dowty *et al.* (*Proc. Natl. Acad. Sci.* (1995) 92:4572-76), Bordignon *et al.* (*Science* (1995) 270:470-75), and Dean (*Exper. Cell Res.* (1997) 230:293-302). Each of these references is supplied in the Supplemental Information Disclosure Statement filed on the same day herewith.

An examination of each of the references cited in Felgner *et al.* provides no evidence that labeling methods either damage DNA or diminish gene expression. Loyter *et al.* is directed to the mechanisms by which calcium phosphate co-precipitation facilitates DNA uptake into cells. The DNA was ^{3}H-labeled; however, gene expression of the labeled DNA was not examined, nor is there any evidence of DNA damage of the labeled DNA. Tsuchiya *et al.* uses ^{3}H and biotin-labeled DNA to monitor DNA uptake into isolated yeast nuclei. This reference does not assess gene expression and there is no evidence of DNA damage from labeling. Zabner *et al.* examines plasmid DNA transfer by cationic lipids. The DNA labeled with ethidium bromide was shown to express a

luciferase transgene in proportion to labelled DNA uptake (*see*, for example, page 19000 column 1, first full paragraph and also Figure 3, page 19001). Dowty *et al.* describes microinjection of DNA into rat myotubes to elucidate the mechanism of DNA nuclear transport. Biotin and gold-labelled DNA was used to assess DNA localization; however, no gene expression was assessed with labeled DNA nor was any DNA damage noted. Bordignon *et al.* describes gene therapy in lymphocytes and bone marrow. Labelled DNA was not used in these studies; rather, the transferred DNA was detected by PCR amplification. Dean describes *in situ* techniques for DNA localization studies. This reference does not use labeled DNA for delivery.

Thus, none of the references cited by Felgner *et al.* evidence unpredictability of labeling and gene expression. Besides the references discussed above, the only other methods cited within Felgner *et al.* are peptide nucleic acid labeling methods (page 2, lines 4-32). Since Felgner *et al.* demonstrates the successful use of this technique to label plasmid DNA and obtain gene expression, it is assumed these are not the technologies to which the assertion as to unpredictability refers.

The Office Action also points to the statement of Zelphati *et al.* that states:

> The methods that have been employed to directly modify DNA either reduce or destroy its ability to be transcribed. In addition, the available approaches to chemically modify plasmid, which utilize photolysis, nick translation, or the use of chemically active nucleotides analogs, attack the DNA randomly so that the final product is chemically heterogeneous and poorly defined.

First, as with Felgner *et al.*, Zelphati *et al.* is not evidentiary of the state of the art at the time of filing of the instant application. Second, like the statement of Felgner *et al.*, the broad statement of Zelphati *et al.* has no basis. There is no evidence within the publication of Zelphati *et al.* either

demonstrating the inability to obtain gene expression using labeling methods, nor references to such demonstrations to support such a broad generalization.

On the other hand, Applicant has demonstrated that by following the teachings of the specification, one of skill in the art can introduce intact and condensed labelled large nucleic acid molecules into cells and measure delivery of nucleic acid and gene expression. The DECLARATION of Sandra Vanderbyl submitted with the previous response filed June 16, 2003, which is incorporated by reference herein, demonstrates operability of the methods, including the combination of the steps of the methods, and also further evidences enablement of the claims as of their effective filing date. The DECLARATION clearly demonstrates that labeled large DNA molecules can be delivered into and expressed in cells. The DECLARATION also demonstrates that the presence of the label does not interfere with gene expression. Therefore, by following the methods provided in the specification, one of skill in the art can deliver into and express labeled large nucleic acid molecules in cells.

The law does not require demonstration of delivery and expression of large nucleic acids containing every possible label incorporated into the nucleic acids by every possible method, as long as Applicant has demonstrated how to carry out the steps of the method as claimed. Further, Applicant is not aware of any requirement under current U.S. patent law specifying that the method must be fully reproducible every time it is practiced. Rather, a patent application satisfies the requirements of 35 U.S.C. § 112, first paragraph, as long as it provides sufficient disclosure, either through illustrative examples or terminology, to teach those of skill how to make and use the claimed subject matter with reasonable, but not undue, experimentation.

Further, Applicant has provided herewith several references published as of the filing date of the instant application that rebut the broad, general statements of Felgner *et al.* and Zelphati *et al.* by demonstrating a variety of labelling methods that are compatible with gene expression.

For example, Leahy *et al.* demonstrates methods were known, as of its publication in 1996, to optimize such labeling procedures to obtain gene expression from labeled DNA that is equivalent to the level of expression from unlabelled DNA. Leahy *et al.* demonstrate that a biotin-labelled plasmid remains biologically active and expresses a chloramphenicol acetyltransferase reporter gene (CAT) when transfected into mouse fibroblast cells (page 547, paragraph spanning columns 1 and 2). Leahy *et al.* further demonstrates that the level of expression of the biotin labeled plasmid can be optimized to the level of unlabelled plasmid by generating plasmids that are labelled in plasmid regions other than the transcription cassette of the vector(*see*, for example, page 547 col. 2, last paragraph, page 548, Figures 4 and 6 and page 550, column 1 first and last full paragraphs). As stated by the authors, "We also showed that the loss of biological activity incurred by covalent derivatization of a plasmid expression vector may be overcome by the construction of large hybrid plasmids in which a large proportion of the transcription cassettes are kept unmodified" (page 550, first full paragraph).

Leahy *et al.* provides successful demonstration of chemically labeling plasmid DNA and obtaining gene expression equivalent to unlabeled DNA. Applicant notes that the publication date of this reference is 1996, available to one of skill in the art as of the filing date of the instant application. Further, such method directly rebuts the broad statements made in Felgner *et al.* and Zelphati *et al.* Leahy *et al.* directly addresses potential problems that are posed by Felgner *et al.* and Zelphati *et al.*, "[we] ask the question whether or not it is possible to covalently attach material to an expression vector and retain transcriptional activity" (page 545, column 2, first full paragraph). The authors then demonstrate a method for covalently labeling DNA with photoactivated biotin that does not interfere with transcription and detection of gene expression. Leahy *et al.* conclude that loss of biological activity by covalent derivatization can be overcome by keeping a large proportion of the transcription

cassettes unmodified (*see*, for example, page 550, column 1, first full paragraph). The authors further state that the method "has general applicability in all situations when loss of activity of an expression vector is incurred" (page 550, column 1, first full paragraph). Thus, the generalization that labeling DNA interferes with transcription was not supported by the art available at the time of filing of the instant application, since the art taught methods to overcome such pitfalls.

Applicant also provides additional references, Wils *et al.* and Zabner *et al.*, which demonstrate techniques of nucleic acid labeling that are compatible with gene expression. For example, Wils *et al.* demonstrates the modification of plasmid DNA by covalently coupling the DNA by triple helix formation and photoactivation with a psoralen-oligonucleotide-peptide conjugate (page 43, § 3.3). The modified plasmid and unmodified control were transfected into cells, and transgene expression was measured. The modified plasmid was not diminished in expression as compared to the unmodified plasmid (*see* for example, Figure 3, page 43). Further, Applicant provides Zabner *et al.*, cited by Felgner *et al.*, which demonstrates that plasmid DNA labelled with ethidium bromide can be delivered to cells and luciferase gene expression obtained from the labelled DNA that is proportional to delivery. Thus, given the availability of many labeling techniques that did not interfere with gene expression, Applicant respectfully submits that the state of the art at the time of filing did not teach that DNA labeling disrupts gene expression, nor that obtaining expression from labeled DNA was "unpredictable".

Conclusion

In light of the extensive teachings and examples in the specification, the high level of skill of those in this art, the knowledge of those of skill in the art, the fact that it is predictable to obtain gene expression from labelled nucleic acid molecules, and the breadth of the claims, it would not require undue experimentation for one of skill in the art to follow the steps of the instant

methods to introduce labelled large nucleic acid molecules into a cell such that they are maintained in an intact and condensed form, and to measure delivery and gene expression therefrom.

In particular, it would not require undue experimentation to label such molecules. Accordingly, a consideration of the factors enumerated in *Ex parte Forman* leads to the conclusion that, based on the disclosure in the specification, undue experimentation would not be required to introduce intact and condensed labelled large nucleic acid molecules into a cell such that the nucleic nacid molecules remain intact and condensed in the cell, measure labelled cells as an indication of delivery of nucleic acid and measure the product of a gene encoded by the labelled large nucleic acid as an indication of gene expression in a cell, as instantly claimed.

DECLARATION

Notwithstanding the above arguments, to evidence that the methods as claimed operate as claimed, a DECLARATION under 37 C.F.R. §1.132 of Sandra Vanderbyl was provided along with the previous response of June 16, 2003, which is incorporated by reference herein.

Also, although this DECLARATION was submitted to rebut the Examiner's assertions of inoperativeness, it also further evidences enablement. It is noted that the level of skill in the biotechnical arts is recognized to be high (see, *e.g.*, *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Further, methods for performing the various steps of the claimed methods, such as labelling nucleic acids, delivering nucleic acids to cells and measuring delivery of and gene expression from the delivered nucleic acids are known to the skilled artisan. Although Ms. Vanderbyl is an inventor of this application, in performing the experiments in the DECLARATION, she followed the teachings in the application. Since those of skill in this art typically have advanced degrees, Ms. Vanderbyl, who has an M.S. degree, is representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol.

The DECLARATION of Sandra Vanderbyl demonstrates that labeled large DNA molecules can be delivered into and expressed in cells. The DECLARATION also demonstrates that the presence of the label does not interfere with gene expression. Therefore, by following the methods provided in the specification, one of skill in the art can deliver into and express labeled large nucleic acid molecules in cells. As discussed above, the law does not require demonstration of every embodiment of the method as long as Applicant has demonstrated how to carry out the steps of the method as claimed.

3. Rebuttal of specific arguments presented in the Final Office Action

Applicant respectfully submits that the above analysis demonstrates that the instant application teaches the methods as claimed and that it would not require undue experimentation to practice the full scope of the methods as claimed. Additionally, Applicant wishes to respond to specific issues raised in the instant Final Office Action.

A) The Office Action alleges that the claims encompass inoperative embodiments and therefore undue experimentation would be required for the skilled artisan to practice the full scope of the claimed methods. Applicant respectfully disagrees.

First, the claims do not encompass inoperative embodiments, since the claims recite that the nucleic acid remains intact and condensed. Further, the Examiner has pointed to a single reference, Neves *et al.*, to demonstrate a less than optimal labeling method for plasmid DNA. There is no evidence that labeling techniques in general are inoperative. To the contrary, as discussed above, the DECLARATION of Vanderbyl demonstrates that by following the teachings of the specification, one of skill in the art can label large nucleic acid molecules and introduce them into cells in a manner that does not interfere with gene expression. Applicant has further cited numerous references demonstrating that the time the instant application was filed, one of the skill in

the art knew how to label nucleic acids using a variety of labeling techniques in a manner that did not interfere with gene expression.

Further, even assuming *arguendo* that Neves *et al.* provides an inoperative embodiment encompassed by the claims, Applicant is not required to design around every inoperative embodiment. If one of skill in the art can distinguish such embodiments without undue experimentation, such embodiments do not constitute non-enablement for the scope of the claim. In re Angstadt, 537 F.2d 498, 502-503, 190 USPQ 214, 218 (CCPA 1976). The nick-translation method of Neves *et al.* generates labelled nucleic acid that is not intact. Such damage can detected by procedures that were routine and standard as of the instant application's filing date. For example, as described in Neves *et al.*, nick translation with fluorophore nucleotides results in relaxed linear plasmid, which can be detected by gel electrophoresis (see Figure 1, and page 54, column 2, paragraph 4).

The Office Action then cites Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409, 414 (1984), for the proposition that if the number of inoperative embodiments become significant, undue experimentation then arises. The Office Action has not, however, provided any evidence of a significant number of inoperative embodiments. Rather, the Office Action cites only a single reference that demonstrates any interference of labelling with gene expression, namely, Neves *et al.*

On the other hand, Applicant has provided a DECLARATION of Vanderbyl demonstrating that by following the teachings of the specification, one of skill in the art can label large nucleic acid molecules, introduce them into cells, and measure gene expression at a level that is comparable to that of unlabeled nucleic acid. Further, Applicant provides numerous references (*e.g.*, Leahy *et al.*, Wils *et al.* and Zabner *et al.*), demonstrating that as of the instant Application's filing date, one of skill in the art could label nucleic acids using a variety of labeling techniques in a manner that did not interfere with gene

expression. Therefore, Applicant respectfully submits that there is no evidence of a significant number of inoperative embodiments of the claimed subject matter that would constitute undue experimentation.

Further, assuming *arguendo* that a small number of inoperative embodiments exist, such embodiments are easily distinguished, and thus do not constitute undue experimentation. The instant application teaches that labeling techniques might impact DNA structure and function and that the DNA may be assessed for its ability to remain intact (page 34, lines 4-13). The application further teaches at lines 10-13, "In a particular exemplary analysis of the stability of artificial chromosomes, *e.g.*, ACes, the chromosomes are exposed to the conditions of interest, *e.g.*, IdU labeling, and analyzed under a fluorescent microscope for the ability to remain intact and condensed after incorporation of nucleotide analogs." Further, at the time of filing the instant application, it was well known in the art how to recognize intact from damaged DNA. As evidenced by the references provided herewith, quick, easy and routine methods for screening DNA for damage were known to one of skill in the art at the time of filing. For example, Salles *et al.* (1999) *Biochemie* 81:53-58, reviews available detection methods for DNA damage that can be formatted in microplate assay. Batel *et al.* (1999) *Analytical Biochemistry* 270: 195-200, describes damage caused by chemical, physical and biological agents and high-throughput methods for their detection. The methods available can be performed on nucleic acids that are in cells or in isolated form. One of skill in the art could readily and quickly detect if a labeling method damaged DNA and therefore easily discard any existing labeling methods that were less than optimal. As cited above, a considerable amount of experimentation is permissible, particularly if it is routine experimentation. Thus, one of skill in the art, could easily and routinely use the available routine methods for testing for DNA damage to assure labelled DNA was intact and condensed for use in cell delivery and gene expression studies.

B) The Office action alleges that Applicant has recited and taught the individual steps of labeling nucleic acid, introducing nucleic acid, detecting nucleic acid and detecting gene expression, but that it is the combination of the steps showing introduction of labeled nucleic acid that is both detectable and competent for gene expression, which is lacking. The Office Action further alleges that Applicant has only enabled such a combination for nucleic acid labeled with bromodeoxyuridine or iododeoxyuridine. Applicant respectfully disagrees with this characterization.

As discussed above, Applicant has taught each of the steps of the instantly claimed methods, as well as the combination of the steps. Applicant has demonstrated successful labeling and detection of gene expression both in the application as filed (*see*, for example, Example 7) and in the DECLARATION of Sandra Vanderbyl. As discussed above, the teachings of the specification, including the exemplification of the methods, are illustrative of the scope of the claims and are thus sufficient to satisfy the enablement requirement of 35 U.S.C. In re Fisher, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). There is no requirement to present a specific example of everything within the scope of a broad claim. In re Anderson, 176 USPQ 331, at 333 (CCPA 1973).

C) The Office Action alleges that because obtaining expression from labelled DNA is unpredictable, that the Applicant must provide additional evidence to overcome this presumption in order to enable the full scope of the claims. Applicant respectfully disagrees.

The Office Action has made an assumption of unpredictability without full consideration of the available data and without providing support for such a broad-sweeping assertion. Applicant respectfully submits that the references cited by the Examiner and provided by Applicant demonstrate successful labeling of nucleic acids in a manner that does not interfere with gene expression. A number of labeling techniques that do not interfere with gene expression, such as those of Zelphati *et al.*, Felgner *et al.*, Wils *et al.*, Zabner *et*

al. and Leahy *et al.*, were available at the time of filing. These references rebut the broad, unsupported generalizations pointed to by the Examiner in Zelphati *et al.* and Felgner *et al.* as demonstrative of the state of the art as of the instant application's filing date.

Further, the working examples, such as Example 7, also clearly demonstrate that labelled nucleic acids are competent for gene expression. Therefore, given the availability of labelling methods in the art which do not interfere with gene expression, and given the specification, which teaches how to introduce intact and condensed labelled large nucleic acid into a cell such that the nucleic acid molecules remain intact and condensed after delivery; measure the delivery of the nucleic acid to the cell by detecting labelled cells; and determine the expression of the product of the reporter gene encoded by the nucleic acid, one of skill in the art could use the available labeling technologies in conjunction with the teachings of the specification to make and use the full scope of the claimed methods.

D) The Office Action contends that Applicant has shown knowledge in the art for the individual steps of the claimed method but the Office Action alleges that it is the knowledge of obtaining expression from a reporter gene from a labeled DNA molecule that is unpredictable, not the knowledge of the individual steps. Applicant respectfully disagrees.

Applicant has provided references that clearly evidence a broad body of knowledge had amassed at the time of filing in the areas of delivery of nucleic acids to cells, in the labeling of nucleic acids, and in the use of reporter genes to measure gene expression from labeled nucleic acids, that could be used with the instantly claimed subject matter. Labeling techniques, such as the labeling methods of Leahy *et al.*, Wils *et al.*, and Zabner *et al.*, provided by Applicant, and Felgner *et al.* and Zelphati *et al.*, cited by the Examiner, were available at the time of filing, which allowed successful gene expression with labeled DNA. Furthermore, it is not the knowledge of those of skill in the art alone, but the

teachings of the specification in conjunction therewith that allow one of skill in the art to bring together the knowledge on delivery methods and gene expression to practice the claimed methods for detecting gene expression from labelled large nucleic acids. As discussed above, Applicant has demonstrated that the teaching of the claimed methods are enabled and operable and that one of skill in the art can use a variety of nucleic acid delivery methods and reporter genes in the art to obtain gene expression from labelled nucleic acids.

E) The Office Action alleges that the state of the art teaches the unpredictability of obtaining gene expression from labeled DNA as evidenced by Felgner *et al.* and Zelphati *et al.*

Although each of the above cited references provides an example of labeling plasmid DNA with peptide nucleic acid in a manner that results in successful detection of gene expression from the plasmid, the Office Action states that Felgner *et al.* and Zelphati *et al.* are not cited for their successful gene expression from labeled DNA but rather it is their summary of the pitfalls of particular techniques for which they are cited (Office Action at page 14).

First, Applicant respectfully submits that one can not pick through a reference and selectively interpret its teachings. As is acknowledged at page 15 of the Office Action, Zelphati *et al.* describes a successful method for obtaining gene expression from labeled DNA. Second, as discussed above, Felgner *et al.* and Zelphati *et al.* provide no support for the general statements therein as to the state of the art at the time the instant application was filed. None of the references cited by Felgner *et al.* and Zelphati *et al.*, in the context of the broad statements made therein, demonstrate any interference of gene expression by virtue of labeling nucleic acids. In fact, one of the references, Zabner *et al.*, actually rebuts such assertions by demonstrating gene expression from ethidium-labeled DNA. When considered together, the references cited by the Examiner and provided and discussed by Applicant above, including Felgner *et al.*, Zelphati *et al.*, Zabner *et al.*, Leahy *et al.* and Wils *et al.*, evidence the

success rather than the inoperability of labeling methods that were compatible with gene expression as of the instant Application's filing date. Applicant respectfully submits that it is the state of the art **at the time of filing** that must be considered in an assessment of enablement, not the state of the art of an earlier period to which Zelphati *et al.* and Felgner *et al.* refer in their introductory paragraphs.

In light of the above, it is respectfully submitted that the instant specification teaches one of skill in the art to make and use the claimed subject matter, namely, how to prepare intact and condensed labelled large nucleic acids, how to introduce intact and condensed labelled large nucleic acids such that the nucleic acid remains intact and condensed after delivery, how to detect labelled cells as an indication of delivery and how to measure the product of a reporter gene encoded by the nucleic acid as an indication of DNA expression. The specification and working examples teach the combination of the steps of the methods as well as detail each of the steps of the methods.

Further, as discussed above, the state of the art at the time of filing demonstrates successful gene expression from labelled nucleic acids introduced into cells, and includes teachings of how to avoid, by routine experimentation, the pitfalls of nucleic acid damage sustained as a result of labeling.

The instant application also demonstrates that by using the methods provided and claimed, one of skill in the art can label large nucleic acids, introduce them into cells and obtain gene expression from the labeled nucleic acids. Therefore, Applicant respectfully submits it would not require undue experimentation to introduce intact and condensed labelled large nucleic acid into a cell such that the nucleic acid remains intact and condensed after delivery, measure labelled cells as an indication of nucleic acid delivery, measure the product of a gene encoded by the nucleic acid as an indication of gene expression in the cell, irrespective of the method for labelling the nucleic acid molecules. Further, given the availability of successful labeling methods and the

teachings of the instant application with respect to introducing intact and condensed nucleic acids into a cell and obtaining gene expression therefrom, Applicant respectfully submits that the claims are commensurate in scope with Applicant's discovery and its disclosure in the instant application.

Policy Considerations

Also, since it is known that a variety of nucleic acid delivery agents and nucleic acid labelling agents and techniques were available as of the instant application's filing date for use in the methods as instantly claimed, it would be unfair and unduly limiting to require Applicant to limit these claims to a method in which the nucleic acid labelling reagent is only one of two agents, namely, BrdU or IdU. To do so is contrary to the public policy upon which the U.S. patent laws are based. If Applicant is required to limit the claims to only the aforementioned labelling reagents, then those of skill in the art could by virtue of the teachings of this application readily practice what is claimed by substituting another labelling reagent and practice what is disclosed in the application, but avoid infringing such limited claims. To permit that is simply not fair. The instant application exemplifies delivery of labelled large nucleic acids into cells and expression of genes encoded by the labelled large nucleic acids in the cells. The executed DECLARATION filed along with the response of June 16, 2003, further demonstrates that it is possible to follow the teachings of the specification to obtain delivery and expression of a labelled large nucleic acid molecule in a cell such that the level of expression is comparable to that obtained using unlabelled nucleic acid. Having done so, it is now routine for others to use other well-known nucleic acid labelling reagents and/or delivery agents in the instantly claimed methods. Those of skill in the art should not be permitted to make such minor modifications by substitution of a different host and avoid infringing such claims.

THE REJECTION OF CLAIMS 1-4 and 11-15 UNDER 35 U.S.C. §103(a)

Claims 1-4 and 11-15 are rejected under 35 U.S.C. §103(a) as unpatentable over Felgner *et al.* (International PCT application No. WO 99/13719) or Zelphati *et al.* ((1999) *Hum. Gene Therap.* 10:15-24) in view of Nolan *et al.* (International PCT application No. WO 00/34436). The Office Action alleges that Felgner *et al.* and Zelphati *et al.* teach delivery and expression of labeled DNA molecules. The Office Action further alleges that Nolan *et al.* teaches delivery of fluorescently labelled large DNA molecules into cells, where the large DNA molecule includes natural chromosomes and artificial chromosomes. The Examiner concludes that it would have been obvious to one of ordinary skill in the art to modify the methods taught by Felgner *et al.* and Zelphati *et al.* according to the teachings of Nolan *et al.* to deliver fluorescently labelled transcriptionally active chromosomes into cells. The Office Action supports this assertion by stating that one of ordinary skill in the art would have been motivated to combine the teachings of Felgner *et al.* or Zelphati *et al.* and those of Nolan *et al.* because Nolan *et al.* states that "the introduction of intact single chromosomes into cells offers unprecedented usefulness as a...method for generating transgenic animals" and Zelphati *et al.* allegedly teaches that their methodology provides a means to tag DNA without disrupting the structural or functional integrity of the DNA. Reconsideration of this rejection is respectfully requested in view of the amendments herein and the following remarks.

The Claims

Claim 1 recites a method for detecting or determining delivery and expression of a nucleic acid introduced into a cell by introducing intact and condensed labelled large nucleic acid molecules that encode a reporter gene into cells such that the introduced nucleic acid molecules remain intact and acid into a cell and measuring the product of the reporter gene as an indication of DNA expression, whereby delivery and gene expression is determined or detected. The dependent claims further specify methods of detection, types of nucleic

acids and cells, delivery methods and labelling methods. New Claim 33 specifies that the nucleic acid is labeled by metabolic or *in vivo* labeling.

Teachings of the cited art and the differences between the teachings of the cited art and Claims 1-4 and 11-15

Felgner *et al.*

Felgner *et al.* teaches methods for introducing plasmid DNA hybridized in a sequence-specific manner to labeled peptide nucleic acid (PNA), including rhodamine-PNA conjugates, into cells and measuring gene expression from these plasmids.

Felgner *et al.* does not teach or suggest methods for the delivery of large labelled nucleic acid molecules. The instant application defines "large" nucleic acids as being "at least about 0.5 megabases" (*i.e.*, about 500 kB) (page 9, line 30 through page 10, line 1). The plasmid DNA molecules taught by Felgner *et al.* are of the order of less than 10 kilobases (*see* Example 1 beginning at page 12 and references incorporated therein).

Felgner *et al.* also does not teach or suggest the introduction of intact and condensed large nucleic acid molecules into cells, nor the introduction of nucleic acid molecules into cells such that the nucleic acids remain intact and condensed after delivery. Unlike the instant methods, the methods of Felgner *et al.* introduce plasmid DNA into cells without any condensation. The plasmid DNA is contacted with PNA and then introduced into the cell (*see*, for example, page 3, lines 21-28; Example 6, page 16, lines 15-24). With respect to Claim 5 and new Claim 33, Felgner *et al.* also does not teach or suggest any metabolic or *in vivo* labeling of the nucleic acid, nor the use of bromo- or iodo-deoxyuridine to effect such labeling.

Zelphati et al.

Zelphati *et al.* teaches a method of hybridizing rhodamine-labelled peptide nucleic acid molecules (PNA) to plasmid DNA encoding a green fluorescent protein (GFP) reporter gene and detection of the labelled plasmid and GFP in cells.

Zelphati *et al.* does not teach or suggest the introduction of labelled large nucleic acids into cells. The plasmids used in Zelphati *et al.* range in size from about 5 kB to about 8 kB (page 2, column 2, first paragraph). Zelphati *et al.* also does not teach or suggest methods of introducing intact and condensed labelled large nucleic acid molecules into cells, nor methods where the nucleic acid molecules remain intact and condensed after delivery. Further, with respect to Claim 5 and new Claim 33, Zelphati *et al.* does not teach or suggest any metabolic or *in vivo* labeling of the nucleic acid, nor the use of bromo- or iodo-deoxyuridine to effect such labeling.

Nolan et al.

Nolan *et al.* teaches methods and an apparatus that employ fluorescence activated cell sorting (FACS) to process large numbers of cells in a chromosome insertion protocol. Nolan *et al.* teaches subjecting a cell to a laser pulse light under conditio s sufficient to form a transient hole in the cell plasma membrane, then introducing a single chromosome into the cell through the hole. Nolan *et al.* further teaches that FACS can be employed to confirm the insertion of a single chromosome within a cell.

Nolan *et al.* does not teach or suggest the introduction of labelled large nucleic acid molecules encoding a reporter gene into cells, nor measurement of reporter gene product as an indication of gene expression. Nolan *et al.* also does not teach methods of introducing intact and condensed labelled large nucleic acid molecules where the nucleic acid molecules remain intact and condensed after delivery. While Nolan *et al.* may mention the usefulness of introducing intact chromosomes in a method for generating transgenic animals, Nolan *et al.* does not provide any teaching or suggestion of the expression of genes encoded by intact and condensed nucleic acid molecules in cells. In fact, Nolan *et al.* does not provide any teaching or suggestion regarding gene expression from nucleic acid molecules that are introduced into cells, much less that the nucleic acid molecules expressing the genes are introduced into and

maintained in the cells in an intact and condensed state. Nolan *et al.* also does not teach or suggest a method in which condensed nucleic acid is introduced into a cell and maintained in a condensed state. Further, with respect to Claim 5 and new Claim 33, Nolan *et al.* does not teach or suggest any metabolic or *in vivo* labeling of nucleic acids, nor the use of bromo- or iodo-deoxyuridine to effect such labeling.

ANALYSIS

The combination of teachings of Felgner *et al.* or Zelphati *et al.* with the teachings of Nolan *et al.* does not result in the instantly claimed methods.

None of the cited references, singly or in any combination, teaches or suggests a method for the introduction of intact and condensed labelled large nucleic acid molecules encoding a reporter gene, where the introduced nucleic acid molecules remain intact and condensed after delivery, and nucleic acid delivery and gene expression are detected. As discussed above, neither Felgner *et al.* or Zelphati *et al.* teach or suggest the introduction of intact and condensed labelled large nucleic acid molecules into a cell, nor maintenance of the intact and condensed state of the nucleic acids once they have entered the cell. First, Felgner *et al.* and Zelphati *et al.* describe the introduction of plasmid DNAs, on the order of 10 kB or smaller, into cells. The references are directed to the introduction of small DNAs and do not teach or suggest methods for the introduction of large nucleic acid molecules into cells. Further, neither reference describes the use of intact and condensed nucleic acid for delivery into cells, nor maintenance of intact and condensed nucleic acid molecules in the cell after delivery. Although both Felgner *et al.* and Zelphati *et al.* examine the stability of PNA-plasmid hybrids after labeling, there is no teaching or suggestion that the labelled DNAs are condensed, nor that condensed nucleic acid molecules are used for delivery to cells, nor that gene expression is detected from nucleic acid molecules that are maintained in a condensed state once they are introduced into the cells. The references also do not teach or suggest how to deliver the labelled DNAs to cells such that they remain intact and condensed.

Additionally, because Felgner *et al.* and Zelphati *et al.* are focussed on small DNAs, they do not teach the determination or detection of gene expression from large labelled nucleic acid molecules.

Nolan *et al.* does not cure these deficiencies, since it also does not teach or suggest any methods in which intact and condensed nucleic acid molecules are introduced into cells in a manner that retains the intact and condensed structure of the nucleic acids after delivery into the cells. Further, because Nolan *et al.* does not teach or suggest any measurement of gene expression, much less gene expression from labelled large nucleic acid molecules that are introduced into cells, there cannot possibly be any teaching or suggestion in Nolan *et al.* that the labelled large nucleic acid molecules that express encoded genes are in an intact and condensed state after delivery into the cells. Nolan *et al.* teaches FACS methods that can be used to verify chromosome insertion into a cell, but Nolan *et al.* does not teach or suggest that the chromosome is intact and condensed, nor is there any teaching or suggestion that the chromosomes are maintained intact and condensed after introduction of the chromosomes into a cell.

Therefore, none of the cited references, singly or in any combination, provides any teaching or suggestion of a method in which intact and condensed large labelled nucleic acids are delivered into cells such that they remain intact and condensed, the delivery is monitored by detecting labelled cells, and expression of a reporter gene encoded by the large labelled nucleic acid molecules is measured.

Furthermore, none of the references, singly or in combination, provides any teaching or suggestion of the use of intact and condensed nucleic acids to deliver labelled large nucleic acids into cells. None of the references, singly or in any combination, teaches or suggests that to introduce labelled large nucleic acids into cells and obtain gene expression therefrom, it is desirable to use

intact and condensed nucleic acids and further to introduce them into cells in a manner that retains their intact and condensed structure.

The Office Action alleges that it would be obvious that the labelling and expression taught by Felgner *et al.* and Zelphati *et al.* could be adapted for use with the chromosomes of Nolan *et al.* Applicant respectfully submits, however, that "obvious to try" does not equate to obviousness. The prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done. *Stratoflex Inc. v Aeroquip Corp.*, 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). In addition, the mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ 1783 (Fed. Cir. 1992).

It is respectfully submitted that the Examiner has failed to provide any objective basis in the cited references or general knowledge for a suggestion to do that which Applicant has done. Indeed, courts have reversed obviousness rejections when there was no finding as to the principle or specific understanding within the knowledge of a skilled artisan to make the claimed invention. *In re Kotzab*, 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1318 (Fed. Cir. 2000).

Because there is no evidence of any suggestion in any of the cited references for modifying labelling techniques so they are applicable to large nucleic acid molecules, much less doing so in a manner that maintains the intact and condensed structure of the large nucleic acids, the mere combination of elements of the cited references does not lead to the instantly claimed methods of introducing intact and condensed labelled large nucleic acid molecules into cells, maintaining the intact and condensed structure in the cells, and detecting their delivery and expression therein. Therefore, the Examiner has failed to establish a *prima facie* case of obviousness.

There is no teaching or suggestion in any of the cited references, singly or in combination, of how to label large nucleic acids (> 0.5 Mb) in a manner that does not interfere with the expression of genes encoded by the large nucleic acids. Further, even assuming *arguendo* that large nucleic acid molecules could be successfully labelled by combining the teachings of Felgner *et al.*, Zelphati *et al.* and Nolan *et al.*, there is no teaching or suggestion in any of the references, singly or in any combination, of labeling and introducing intact and condensed large nucleic acid molecules into cells such that they remain intact and condensed after delivery into the cells. The combination of references also does not teach or suggest labelled large nucleic acid molecules that remain intact and condensed, whereby both delivery and gene expression are measured. None of the references alone or in combination teaches or suggests determination or detection of gene expression from labelled large nucleic acids. Nolan *et al.* does not teach or suggest any measurement of gene expression. Felgner *et al.* and Zelphati *et al.* only demonstrate expression from small labelled plasmids.

The Office Action contends that there is no reason to believe that the small DNAs taught by Felgner *et al.* and Zelphati *et al.* are any different from the chromosomes of Nolan *et al.* As Nolan *et al.* points out, however, small and large DNAs can differ; for example, delivery of chromosomes, due to their size, is very difficult by any method and that methods used for smaller DNAs, might not be suitable for large nucleic acids (*see*, for example, page 1, lines 21-24). There is no teaching or suggestion in the cited references, singly or in any combination, that techniques for labelling nucleic acids, introducing labelled nucleic acids into cells and measuring gene expression from labelled nucleic acids that are suitable for small DNAs would be adaptable to large nucleic acids. Further, none of the cited references, singly or in any combination, teaches or suggests introducing labelled nucleic acids into cells in an intact and condensed state, and maintaining the intact and condensed state after delivery into the cells.

In summary, the combination of the teachings of Felgner *et al.* or Zelphati *et al.* with Nolan *et al.* does not result in the instantly claimed subject matter. None of the references, singly or in any combination, teaches or suggests the methods as claimed, which include steps of: introducing intact and condensed labelled large nucleic acid molecules that encode a reporter gene into cells where the nucleic acids molecules remain intact and condensed after delivery; detecting labelled cells as an indication of delivery of the nucleic acid into the cell; and measuring the product of the reporter gene as an indication of DNA expression. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

PROVISIONAL REJECTION OF CLAIMS 1-16 AND 30 UNDER STATUTORY TYPE (35 U.S.C. § 101) DOUBLE PATENTING

Claims 1-16 and 30 are provisionally rejected under statutory type (35 U.S.C. § 101) double patenting as allegedly unpatentable over claims 1-8, 11-16 and 30 of copending U.S. Application No. 10/086,745. Specifically, it is alleged that Claims 1-8, 11-16 and 30, of copending U.S. Application No. 10/086,745, are identical in scope to the claims of the instant application.

An Office Action on the merits recently issued in connection with copending U.S. Application No. 10/086,745. Applicant respectfully submits that when a response is filed thereto, claims of identical scope to the instantly pending claims, if any, will be cancelled or amended to obviate any statutory double patenting issues.

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In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
FISH & RICHARDSON P.C.

By: _____
Stephanie Seidman
Registration No. 33,779

Attorney Docket No. 24601-416B
Address all correspondence to:
Stephanie Seidman, Ph.D, J.D.
FISH & RICHARDSON P.C.
12390 El Camino Real
San Diego, CA 92130
Telephone: (858) 678-5070
Facsimile: (202) 626-7796
email: seidman@fr.com